Cerebellar GABAergic progenitors adopt an external granule cell-like phenotype in the absence of Ptf1a transcription factor expression

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Edited by Lily Y. Jan, University of California, San Francisco School of Medicine, San Francisco, CA, and approved January 23, 2007 (received for review July 10, 2006)

We report in this study that, in the cerebellum, the pancreatic transcription factor Ptf1a is required for the specific generation of Purkinje cells (PCs) and interneurons. Moreover, granule cell progenitors in the external GCL (EGL) appear to be unaffected by deletion of *Ptf1a*. Cell lineage analysis in *Ptf1a*$^{Cre/Cre}$ mice was used to establish that, in the absence of *Ptf1a* expression, ventricular zone progenitors, normally fated to produce PCs and interneurons, aberrantly migrate to the EGL and express typical markers of these cells, such as Math1, Reelin, and Zic1/2. Furthermore, these cells have a fine structure typical of EGL progenitors, indicating that they adopt an EGL-like cell phenotype. These findings indicate that Ptf1a is necessary for the specification and normal production of PCs and cerebellar interneurons. Moreover, our results suggest that Ptf1a is also required for the suppression of the granule cell specification program in cerebellar ventricular zone precursors.

cerebellum | GABAergic cells | neural specification

The cerebellar cortex essentially comprises three major types of neurons: Purkinje cells (PCs), granule cells, and several types of interneurons that include basket, stellate, and Golgi cells (1). PCs and interneurons are GABA-releasing inhibitory neurons, whereas granule neurons use glutamate as their transmitter. A crucial issue in neural development is the identification of the mechanisms by which distinct types of neurons are specified and settle in their correct layers. In the mouse, PCs arise at the ventricular zone (VZ) at embryonic day (E)11–E13 and migrate radially to form the PC layer (PCL). Granule cell progenitors migrate tangentially from the rhombic lip (RL) to form the external granule cell layer (EGL). At postnatal stages, EGL precursors expand dramatically to ultimately migrate inwards and form the granule cell layer (GCL) (2–5). Cerebellar interneurons stem from the VZ and remain mitotically active in the white matter to finally give rise to diverse types of interneurons at postnatal stages (6, 7). It has been shown that the deep cerebellar nuclei and unipolar brush cells originate in the RL (4, 8, 9).

In the cerebellum, the transcription factors *Math1* and *NeuroD* are essential for the specification and generation of glutamatergic granule cells (4, 10, 11), but little is known about the genes that control PC specification. The *Ptf1a* gene encodes a basic helix–loop–helix transcription factor, which is required for the specification and formation of the pancreas (12, 13). A recent study has shown that a 300-kb deletion in the enhancer of the *Ptf1a* gene leads to abnormal cerebellar development as well as loss of PCs and interneurons (7). Ptf1a is also required for the generation of dorsal horn GABAergic interneurons in the spinal cord, and, in its absence, *Ptf1a*-derived cells adopt a glutamatergic phenotype (14). A recent study in the retina shows that inactivation of *Ptf1a* leads to a fate switch in horizontal and amacrine cell precursors that causes them to adopt a ganglion cell fate (15). We have examined the role of *Ptf1a* in the development of the cerebellum by analyzing the phenotype of mice lacking the complete *Ptf1a* coding sequence (*Ptf1a*^{Cre} knockin). We confirm that *Ptf1a* is required for the generation of PCs and interneurons. Moreover, by using cell lineage analysis employing *Ptf1a*$^{Cre/Cre}$*;R26R* mice, we show that, in the absence of *Ptf1a*, progenitors fated to produce PCs and interneurons become incorporated in the EGL and express typical markers of granule cells. These findings indicate that *Ptf1a* is not only required for the specification of PCs and interneurons, but also that Ptf1a expression suppresses a granule cell phenotype from the cerebellar VZ.

Results

***Ptf1a* Is Expressed in PC and Interneuronal Progenitors.** To determine the cell lineages arising from cells in which the *Ptf1a* locus had ever been activated, we examined the cerebella of *Ptf1a*$^{Cre/+}$ mice crossed with *R26R* reporter mice (*Ptf1a*$^{Cre/+}$*;R26R*). X-Gal staining was strong in the brainstem, spinal cord, and developing cerebellar anlage (Fig. 1*A*). At E12, cells located just above the VZ were labeled in the cerebellum [see supporting information (SI) Fig. 7*A*]; at E14, labeling was detected throughout the cerebellar plate (data not shown); and at E18, β-Gal activity was detected around the PCL and deeper in the cerebellar parenchyma (SI Fig. 7*B*). In contrast, no staining was observed in either the RL or the EGL at any stage. Using RT-PCR, we found that cerebellar *Ptf1a* mRNA levels were high at E12, decreased until postnatal day (P)2, and were undetectable in the adult (SI Fig. 7*C*). By *in situ* hybridization(ISH), *Ptf1a* mRNA was detected at E12–E14 in the VZ of the cerebellar anlage containing progenitor cells (Fig. 1*B*). At later stages (E16–P5), mRNA was detected in single cells embedded in the cerebellar parenchyma, most notably below the PCL. No expression was detected in either the EGL or the PCL at any stage analyzed (Fig. 1*C*). To substantiate this expression pattern, sections were immunolabeled with anti-Ptf1a antibodies (16). At E14–E16, Ptf1a was

Author contributions: F.X.R. and F.S. contributed equally to this work; M.P., I.A., A.M.-L.M., F.X.R., and E.S. designed research; M.P., I.A., A.M.-L.M., A.M., and J.A.D.R. performed research; J.A.D.R. and C.V.E.W. contributed new reagents/analytic tools; M.P., I.A., A.M.-L.M., A.M., J.A.D.R., C.V.E.W., F.X.R., and E.S. analyzed data; and M.P., I.A., F.X.R., and E.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS direct submission.

Abbreviations: Calb, calbindin; E(*n*), embryonic day (*n*); EGL, external granule cell layer; PC, Purkinje cell; PCL, PC layer; P(*n*), postnatal day (*n*); RL, rhombic lip; VZ, ventricular zone.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0605699104/DC1.

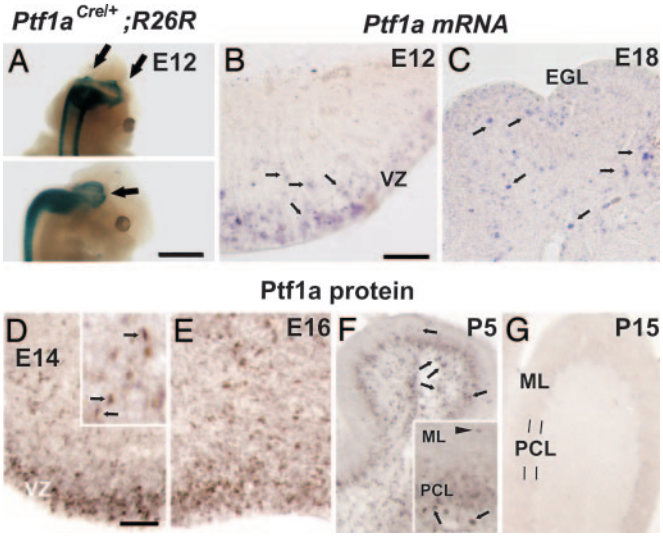

Fig. 1. *Ptf1a* locus activity and expression in the developing cerebellum. (*A*) Lineage tracing analysis of *Ptf1a* activation in the cerebellum of *Ptf1a*$^{Cre/+}$;*R26R* embryos revealed by X-Gal staining. *In toto* labeling showing strong X-Gal staining in the cerebellar plates (arrows) of E12 embryos. (*B* and *C*) *Ptf1a* mRNA expression in the cerebellum. ISH for *Ptf1a* mRNA. (*B*) At E12, the hybridization signal was detected (arrows) at the proliferating VZ and in individual migrating neurons. (*C*) At E18, the hybridization signal was detected in scattered single cells (arrows) throughout the cerebellar parenchyma. (*D–G*) Immunohistochemical expression of Ptf1a. (*D*) Ptf1a localized within the VZ and in migrating postmitotic cells (arrows) at E14. (*E*) At E16, a similar pattern of staining of migrating cells is observed. (*F*) At P5, migrating interneurons expressing Ptf1a (arrows) were observed below the PCL, whereas PCs and granular cells were nonreactive. A Ptf1a-positive interneuron having reached the molecular layer (ML) is identified with an arrowhead. (*G*) Ptf1a was not detected in the cerebellum of P15 mice. [Scale bars: (*A*) 50 μm; (*B*) 50 μm, represents 70 μm in *C*; (*D*) 50 μm, pertains to *E* and represents 100 μm in *F* and *G*.]

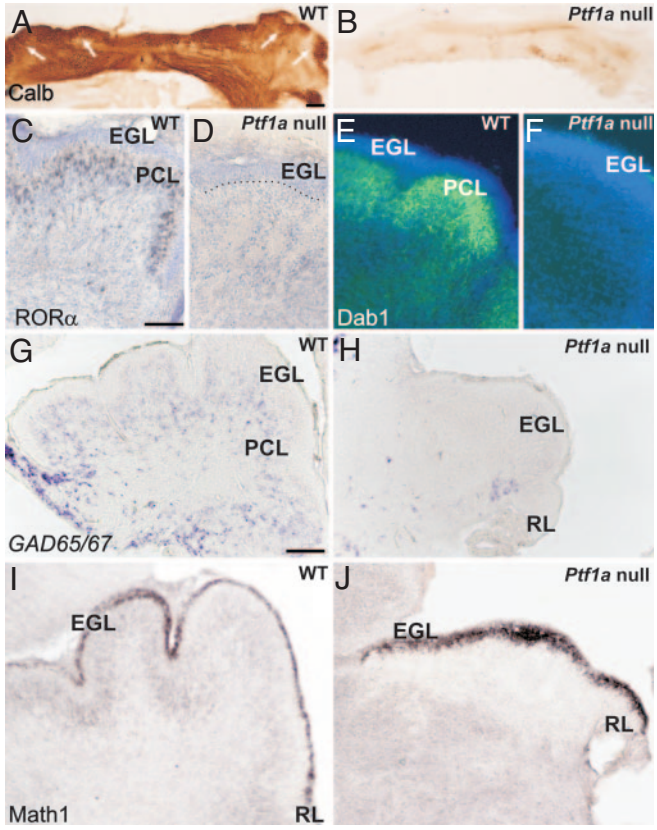

Fig. 2. Cerebellar histology in wild-type and *Ptf1a* null E18 embryos. (*A* and *B*) Coronal sections immunostained for Calb. At E18, the Calb-positive PCL (arrows in *A*) was absent in *Ptf1a* null embryos (*B*). (*C–F*) Immunolabeling for the PC markers ROR-α and Dab1 at E18. Note that ROR-α and Dab1-positive PCs were absent in sections from *Ptf1a* null embryos. (*G* and *H*) ISH for *GAD65*/*GAD67* mRNA showed a dramatic reduction of expression in the mutant cerebella at E18. (*I* and *J*) Sagittal sections immunostained for Math1 in E18 wild-type (*I*) and *Ptf1a* null (*J*) embryos. As in wild-type embryos, cells in the EGL of *Ptf1a*-deficient mice express the typical granule cell marker Math1. [Scale bars: (*A*) 100 μm, pertains to *B*; (*C*) 50 μm, pertains to *D–F*; (*G*) 100 μm, pertains to *H–J*.]

detected in the nuclei of many cells located in the upper part of the VZ (Fig. 1*D*). In addition, single cells exhibiting elongated nuclei, typical of migrating neurons, were immunostained just above the VZ at E14–E16 (Fig. 1*D Inset* and *E*). At E18–P5, Ptf1a was detected in single cells located in the developing white matter, the GCL, and around PCs (Fig. 1*F*). The distribution of these cells resembled that of cerebellar interneurons migrating toward the molecular layer (Fig. 1*F Inset*). Ptf1a was not detected in PCs in the PCL at any developmental stage, nor was it detected in the cerebellum at P15 (Fig. 1*G*) or in adult mice (data not shown). These results suggested that Ptf1a is expressed in a short temporal window in postmitotic PCs and in interneuronal progenitors.

The fate of cells that activate the *Ptf1a* locus was analyzed in *Ptf1a*$^{Cre/+}$;*R26R* mice (12). Lineage tracing in these mice confirmed that, at E18, *Ptf1a*-derived cells (expressing β-Gal) populated the PCL and the cerebellar parenchyma but not the EGL (SI Fig. 8 *A–C* and Fig. 3*A*). Double labeling with β-Gal and calbindin (Calb) confirmed that PCs expressed β-Gal (SI Fig. 8 *A–C*). At postnatal ages (P20) and adult, β-Gal was detected in PCs (labeled by Calb) (SI Fig. 8 *D–F*) and in parvalbumin-labeled interneurons of the molecular layer, but not in the GCL (SI Fig. 8 *H–J*) or in glial fibrilary acidic protein (GFAP)-immunoreactive cells (SI Fig. 8*G*). These lineage analyses indicated that *Ptf1a*-expressing cells selectively give rise to PCs and interneurons.

***Ptf1a* Is Required for the Development of PCs and Interneurons.** To examine the *in vivo* role of *Ptf1a*, we analyzed the phenotype of *Ptf1a* null embryos (*Ptf1a*$^{Cre/Cre}$). Mice lacking Ptf1a expression die at birth (12, 13), so only embryos up to E18 were analyzed. Histological examination of coronal and sagittal sections at E14–E18 revealed cerebellar hypoplasia in mutant embryos (SI Fig. 9 *A* and *B*). At E14, Calb was detected in a broad band of migrating PCs, which were more abundant in the caudal half of cerebella (SI Fig. 9*C*). *Ptf1a* null embryos showed much lower Calb immunostaining than did wild-type embryos (SI Fig. 9*D*), although there was some interindividual variability: whereas six of eight cerebella exhibited a complete loss of PCs at E14 (data not shown), two embryos had some Calb-positive cells at this age. At E16–E18, the lack of Calb-expressing PCs in mutants was much more dramatic. In contrast to wild-type embryos, PCs were virtually absent from mutant cerebella (Fig. 2 *A* and *B* and SI Fig. 10 *F* and *G*). To further substantiate the lack of PCs, we analyzed the expression of two additional PC markers: Dab1 and RORα (17, 18). In contrast to the controls, *Ptf1a* null cerebella at E18 were devoid of Dab1- and RORα-immunoreactivities (Fig. 2 *C–F*). We found no evidence of ectopic PCs in other brain regions close to the mutant cerebellum.

We next examined whether other neuronal components of the cerebellum were also altered in *Ptf1a* null embryos. GABAergic interneurons and their progenitors, labeled with anti-Pax2 antibodies (19), were also severely compromised in *Ptf1a* null cerebella. At E14, we found one mutant cerebellum exhibiting a complete loss of Pax2 immunoreactivity, whereas in three other mutant embryos, the number of Pax2-positive cells was reduced

by one third (SI Fig. 10 *A–C*). At E16–E18, Pax2-positive interneurons were abundant in wild-type cerebella, but were completely absent in *Ptf1a* null embryos (SI Fig. 10 *D* and *E*). To confirm the lack of GABAergic neurons, we mapped the expression of glutamic acid decarboxylase (*GAD65/67*) mRNA (20). In control mice, at E14, *GAD65/67* transcripts were detected above the VZ and in the cerebellar parenchyma (data not shown); at E16–E18, expression was prominent in cells in the white matter exhibiting the typical distribution of interneuronal progenitors (21) and was faint in PCs (Fig. 2*G*). *GAD65/67* mRNA was virtually undetectable in the cerebella of *Ptf1a* null mice at E14–E18 (Fig. 2*H*). We thus conclude that genetic ablation of *Ptf1a* results in the early loss of PCs and interneurons in the cerebellum.

***Ptf1a* Is Required for the Survival of PCs and Interneurons.** The above findings, in which a more dramatic cerebellar phenotype was observed in E18 embryos, suggested that Ptf1a also has a role in cell survival. To investigate this possibility, sections were immunostained for activated caspase-3 (caspase-3^{A}). In comparison with wild-type embryos, cerebella from *Ptf1a* null mice had significantly higher numbers of capase-3^{A}-positive cells, particularly at E16 and E18 (SI Fig. 11 *A*, *B*, and *F*). Furthermore, in mutant cerebella, many caspase-3^{A}- and Calb-immunoreactive cells were atrophic and exhibited disrupted, beaded dendrites characteristic of dying neurons (Fig. 11 *C–E*). These findings therefore indicated that Ptf1a is also involved in the survival of PCs and interneurons.

EGL Cells Are Spared in P*tf1a* Null Embryos. EGL cell distribution was investigated by using the markers Math1, Netrin-1, Reelin, and Zic1/Zic2 (5, 22–27). At E16–E18, Math1 and Netrin-1 labeled the entire EGL and RL in control cerebella (Fig. 2*I* and SI Fig. 12 *E* and *F*). The EGL/RL was also labeled by Reelin and Zic1/Zic2, which also marked other cerebellar neurons (25–27) (SI Fig. 12 *A* and *C*). A similar expression pattern was observed in *Ptf1a* null embryos for all four EGL markers, although labeled EGL cells appeared to be more densely packed in the EGL of null embryos (Fig. 2*J* and SI Fig. 12 *B*, *D*, *G*, and *H*). Semiquantitative RT-PCR analysis showed a marked down-regulation of the mRNA that correspond to PCs (*Calb* and *Dab1*) and interneurons (*Pax2*) in *Ptf1a* null mice, whereas the mRNA of granule cell markers (*Zic2*, *Pax6*, and *Reelin*) were unaffected (SI Fig. 9*E*). These results support the notion that Ptf1a is not required for EGL cell specification.

***Ptf1a*-Expressing Progenitors Settle in the EGL of *Ptf1a*$^{Cre/Cre}$;*R26R* Null Embryos.** To investigate the fate of progenitors that activate the *Ptf1a* locus in the absence of Ptf1a expression, we studied *Ptf1a*$^{Cre/Cre}$;*R26R* embryos (12). In heterozygous embryos (*Ptf1a*$^{Cre/+}$;*R26R*), β-Gal-expressing *Ptf1a*-derived cells populated the PCL and the cerebellar parenchyma, but not the EGL (Fig. 3*A*; see also SI Fig. 13 *I* and *J*). In contrast, in *Ptf1a*$^{Cre/Cre}$;*R26R* embryos, X-Gal-labeled cells were found both in the cerebellar parenchyma and densely concentrated in the EGL (Fig. 3*B*). The RL was unlabeled in both mouse strains. A serial section analysis at different developmental stages showed that allocation of *Ptf1a*-derived cells to the EGL of *Ptf1a*$^{Cre/Cre}$;*R26R* embryos had already occurred at E14–E16 and was detected along the entire mediolateral extent of the cerebella (SI Fig. 13 *A–L*). Interestingly, β-Gal-expressing cells accumulated in the rostral half of the EGL, whereas these cells were absent from the caudal EGL and the entire RL (Fig. 3*B* and SI Fig. 13 *A–L*).

These results suggested that precursors activating the *Ptf1a* locus in *Ptf1a* null embryos are allocated to the EGL. To confirm this possibility, neurons born at E12 [the day of generation of PCs (3, 28)] were pulse-labeled with BrdU and analyzed at E18. In wild-type embryos, E12-generated neurons were detected in

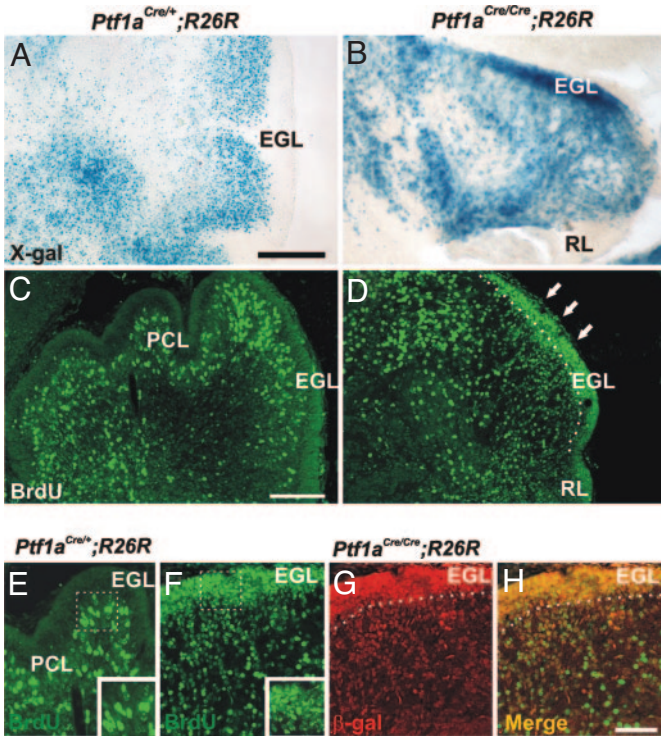

Fig. 3. Lineage tracing of *Ptf1a*-expressing cells and contribution of E12-generated cells to the EGL. (*A* and *B*) Lineage tracing of *Ptf1a*-expressing cells and contribution to the EGL. In *Ptf1a*$^{Cre/+}$;*R26R* mice, cells with an activated *Ptf1a* locus do not contribute to the EGL (*A*). By contrast, the EGL, but not the RL, of *Ptf1a* null embryos is densely populated by cells with an activated *Ptf1a* locus (*B*). (*C* and *D*) In wild-type mice, BrdU-positive cells are found in the region of the PCL and in the parenchyma but not in the EGL (*C*). In contrast, *Ptf1a*-deficient mice display a very high number of BrdU-positive cells in the EGL (*D*, arrows). (*E–H*) In *Ptf1a*$^{Cre/+}$;*R26R* mice, labeled cells are found mainly in the PCL and are absent from the EGL (*E*). In *Ptf1a*$^{Cre/Cre}$;*R26R* cerebella, labeled cells are abundant in the EGL and in the deep layers (*F*). Double immunostaining with antibodies detecting β-Gal showed the presence of BrdU-labeled cells that had activated the *Ptf1a* locus in the EGL (*F–H*). [Scale bars: (*A*) 100 μm, pertains to *B*; (*C*) 100 μm, pertains to *D*; (*E*) 100 μm, pertains to *F–H*.] The EGL is labeled by dashed lines.

the cerebellar parenchyma and along the PCL, whereas the EGL was unlabeled (Fig. 3 *C* and *E*). The distribution of BrdU-labeled cells was dramatically altered in *Ptf1a*-null embryos, in which a high number of E12 BrdU-immunoreactive cells were allocated in the EGL, indicating abnormal migration and positioning (Fig. 3 *D* and *F*). Moreover, whereas in wild-type embryos, BrdU-labeled nuclei in the PCL were large, corresponding to PCs (Fig. 3 *C* and *E*), in *Ptf1a*-null embryos, BrdU-positive nuclei in the EGL were small, resembling EGL cells (Fig. 3 *D* and *F*). To confirm that, in *Ptf1a*$^{Cre/Cre}$;*R26R* embryos, neurons that are generated at E12 and then settle in the EGL are derived from cells having an activated *Ptf1a* locus, sections were immunostained for BrdU and β-Gal. BrdU-stained cells located in the EGL of *Ptf1a*$^{Cre/Cre}$;*R26R* embryos were also immunolabeled for β-Gal (Fig. 3 *F–H*). These data showed that, in *Ptf1a*-null mutants, E12-generated neurons, in which the *Ptf1a* locus had been activated, abnormally migrate to the EGL, suggesting that they might acquire an EGL phenotype.

***Ptf1a*-Expressing Progenitors Acquire an EGL-Like Phenotype in *Ptf1a*$^{Cre/Cre}$;*R26R* Null Embryos.** The phenotype of cells derived from *Ptf1a*-active precursors in *Ptf1a*$^{Cre/Cre}$;*R26R* cerebella was assessed by using double immunofluorescence with several EGL markers and anti-β-Gal antibodies. We first used two EGL cell

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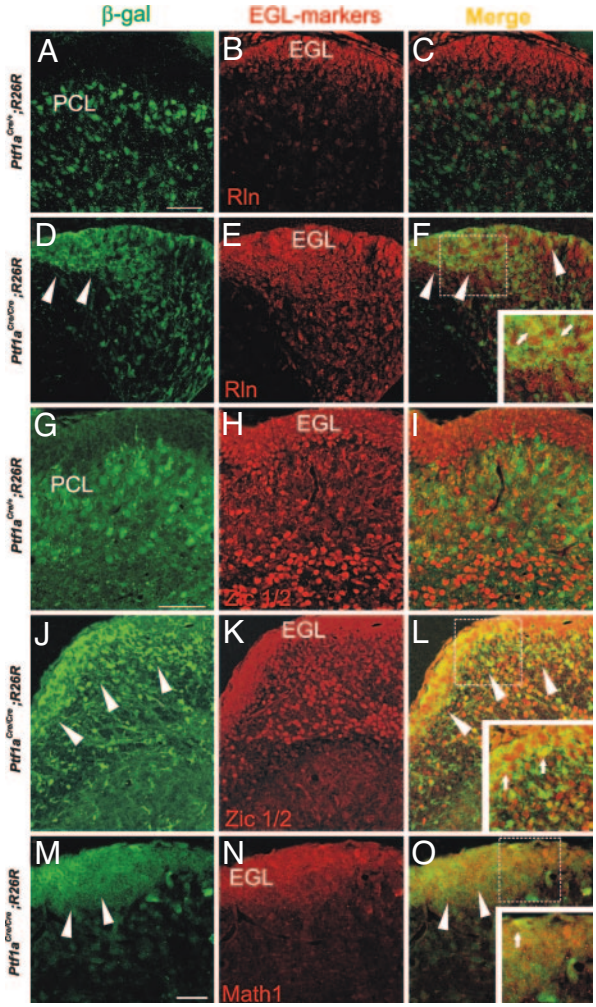

Fig. 4. Cerebellar ventricular zone progenitors lacking *Ptf1a* acquire an EGL-like cell phenotype at E18. (*A–F*) Double-labeling immunofluorescence with antibodies detecting β-Gal and the granule cell marker Reelin in the cerebellum of *Ptf1a*$^{Cre/+}$*;R26R* and *Ptf1a*$^{Cre/Cre}$*;R26R* embryos. In wild-type cerebella, cells with an activated *Ptf1a* locus do not express Reelin and display characteristic distribution of PCs and interneurons (*A–C*). In mutant cerebella, β-Gal-positive cells are found in the rostral EGL (large arrowheads) and coexpress Reelin (*D–F*, arrows). (*G–L*) Double-labeling immunofluorescence with antibodies detecting β-Gal and Zic1/2 in the cerebellum of *Ptf1a*$^{Cre/+}$*;R26R* and *Ptf1a*$^{Cre/Cre}$*;R26R* embryos. In wild-type cerebella, *Ptf1a*-derived cells do not express Zic1/2 and display the distribution of PCs and interneurons (*G–I*). In mutant cerebella, β-Gal-positive cells in the rostral EGL (large arrowheads) coexpress both β-Gal and Zic1/2 (*J–L*, arrows). (*M–O*) β-Gal-positive cells located in the EGL (large arrowheads) of *Ptf1a*$^{Cre/Cre}$*;R26R* mutant embryos are also labeled by Math1 antibodies (arrow). [Scale bars: (*A*) 50 μm, pertains to *B–F* and *J–L*; (*G*) 50 μm, pertains to *H* and *I*; (*M*) 25 μm, pertains to *N* and *O*.]

markers, Zic1/2 and Reelin, which are expressed in granule cell precursors but not in PCs or interneurons (24, 26, 27). At E16–E18, β-Gal was undetectable in the EGL of *Ptf1a*$^{Cre/+}$*;R26R* control cerebella, whereas Reelin and Zic1/2 proteins labeled this cell population; no cells coexpressing these markers were detected in the EGL (Fig. 4 *A–C* and *G–I*). β-Gal was expressed in large neurons of the PCL, as described above. In contrast, in *Ptf1a*$^{Cre/Cre}$*;R26R* null embryos, β-Gal was detected in densely packed cells expressing Reelin and Zic1/2 in the rostral half of the EGL, supporting a granule phenotype for these cells (Fig. 4 *D–F* and *J–L*). The caudal portions of the EGL and the RL were populated by Reelin/Zic1/2-positive cells that lacked β-Gal

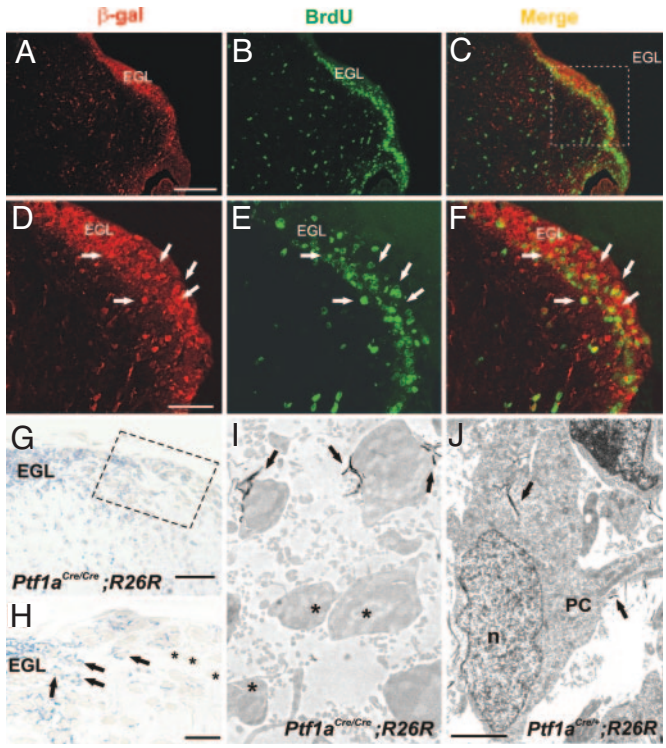

Fig. 5. Proliferation and ultrastructure of *Ptf1a*-derived cells in *Ptf1a*$^{Cre/Cre}$*;R26R* embryos. (*A–F*) Pregnant females were injected with BrdU at E18, and proliferating precursors were analyzed 2 h after the injection. VZ progenitors lacking Ptf1a (β-Gal) and populating the EGL are colabeled by BrdU. Double-labeled β-Gal/BrdU-positive cells in the EGL layer are marked by arrows. (*G–J*) Electron microscopy of β-Gal-expressing cells in *Ptf1a*$^{Cre/Cre}$*;R26R* null mutant and *Ptf1a*$^{Cre/+}$*;R26R* embryos. (*G* and *H*) Semithin sections from a *Ptf1a*$^{Cre/Cre}$*;R26R* embryo illustrating that endogenous (labeled by asterisk) and *Ptf1a*-derived (Bluo-Gal-stained, labeled by arrows) EGL cells have similar sizes. (*I*) Electron micrograph showing that Bluo-Gal-labeled cells (arrows) display a fine structure identical to that of unlabeled EGL cells (asterisk). Electron micrograph illustrating a β-Gal-labeled PC in *Ptf1a*$^{Cre/+}$*;R26R* cerebella. Arrows in *J* label enzymatic reaction end product. [Scale bars: (*A*) 150 μm, pertains to *B* and *C*; (*D*) 50 μm, pertains to *E* and *F*; (*G*) 25 μm; (*H*) 10 μm; (*J*) 2 μm, pertains to *I*.]

expression (Fig. 4 *F* and *L*), indicating that these cells originate from Ptf1a-negative RL.

To further support these findings, sections were immunolabeled with antibodies detecting Math1, which is exclusively expressed in EGL cells and the RL at E16–E18. As expected, in control *Ptf1a*$^{Cre/+}$*;R26R* cerebella, Math1 was detected in EGL cells (see Fig. 2*I*) and did not colocalize with β-Gal-positive PCs and/or interneurons (data not shown). In contrast, in *Ptf1a*$^{Cre/Cre}$*;R26R* null embryos, β-Gal was detected in a densely packed cell population expressing Math1 in the rostral half of the EGL, thereby indicating an EGL-like phenotype for cells derived from *Ptf1a*-active precursors (Fig. 4 *M–O*). Thus, in *Ptf1a*$^{Cre/Cre}$*;R26R* null embryos, *Ptf1a*-derived cells express the EGL cell markers Zic1/2, Reelin, and Math1.

To further substantiate an EGL-like phenotype, β-Gal-expressing cells were analyzed by two additional criteria. Whereas PCs at E18 are postmitotic neurons, EGL precursors are mitotically active cells. Thus, E18 embryos were labeled by BrdU pulses, and proliferating cells were mapped 2 h later. As expected, in *Ptf1a*$^{Cre/+}$*;R26R* control embryos, BrdU-positive cells were detected in the EGL, whereas β-Gal-immunoreactive PCs were BrdU-negative (data not shown). In contrast, in *Ptf1a*$^{Cre/Cre}$*;R26R* null embryos, a subset of β-Gal-immunoreactive cells in the EGL was also labeled by BrdU (Fig. 5 *A–F*), indicating that at least some cells derived from *Ptf1a*-active precursors are mitotically active in null mice.

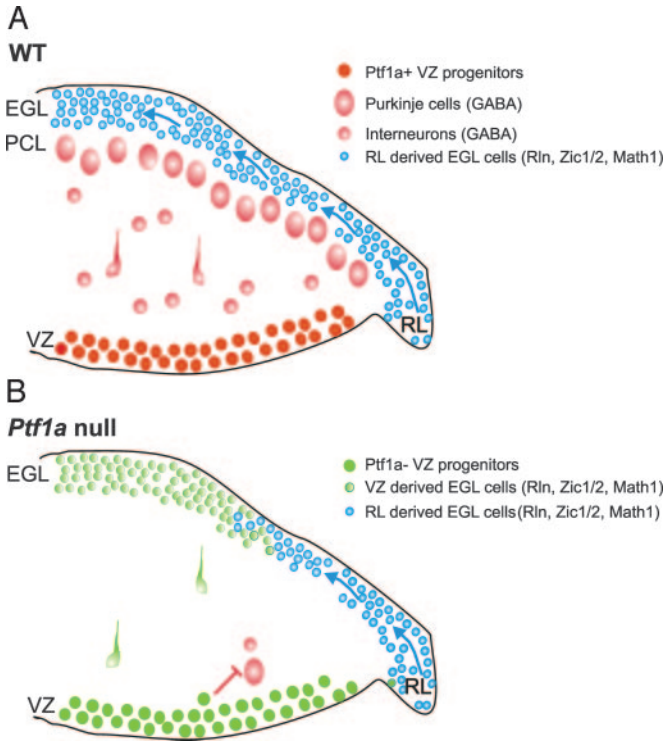

Fig. 6. Role of Ptf1a in neuronal specification in the cerebellum. (*A*) In wild-type mice, Ptf1a-expressing VZ progenitors produce GABAergic cells in the cerebellum, including PCs and interneurons (red). In parallel, RL progenitors produce granular cell precursors located in the EGL expressing Math1, Reelin, and Zic1/2 (blue). (*B*) In absence of *Ptf1a*, VZ precursors are unable to produce functional GABAergic cells but generate small-sized cells expressing Math1, Reelin, and Zic1/2, which abnormally invade the EGL (green). In *Ptf1a* null embryos, the production of granule cells by RL progenitors is preserved. Thereby, the EGL of *Ptf1a* null embryos is populated in part by EGL cells produced normally in the RL (blue) and by cells with an EGL-like phenotype produced by VZ progenitors lacking Ptf1a (green).

Lastly, the fine structure of β-Gal-positive cells was studied at E18. In $Ptf1a^{Cre/+}$;*R26R* embryos, β-Gal-stained cells were large, vertically oriented, and showed the typical fine structure of embryonic PCs (Fig. 5*J*). In null embryos, β-Gal-labeled cells in the EGL were small, oriented parallel to the pia, and had small, condensed nuclei and little cytoplasm (Fig. 5 *G–I*). These fine structural features were identical to those of unlabeled EGL precursors (Fig. 5*I*). Altogether, these findings indicate that, in the absence of Ptf1a, cells that originate in the VZ at E12 shift to a laminar location in the EGL and acquire a gene expression pattern and a morphology characteristic of granule cell precursors.

Discussion

***Ptf1a* Is Expressed in Progenitors of PCs and Interneurons.** *Ptf1a* is expressed in several regions of the developing nervous system (14, 29). We show here that in the cerebellum, *Ptf1a* mRNA and protein are expressed in the VZ at E12–E14 and, at perinatal stages, in white matter cells (presumably interneuronal progenitors). In contrast, *Ptf1a* is undetectable in the RL or in the EGL as well as in the adult. Our lineage tracing analyses in $Ptf1a^{Cre/+}$;*R26R* mice show that cells in which *Ptf1a* is activated give rise to PCs and cerebellar interneurons, including stellate, basket, and Golgi cells, but not to granule cells. These findings confirm and extend previous reports (7). However, we did not detect any *Ptf1a*-derived cells expressing GFAP, unlike Hoshino *et al.* (7).

***Ptf1a* Is Required for the Generation and Survival of PCs and GABAergic Interneurons.** Loss of function of the *Wnt1*, *Fgf8*, *En1/2*, *Gbx1*, and *Pax2* genes, among others, has dramatic effects on cerebellar development, most frequently leading to a complete loss of all major neuronal types (1, 30). Heterotopic and heterochronic grafting experiments have shown that PCs and interneurons are committed to these fates at E12, even when cerebellar progenitors are transplanted to ectopic brain areas (21, 31). Similarly, EGL precursors appear to be restricted to a granule cell phenotype (31–33). These data indicate that cerebellar neuron specification occurs very early and through rigid molecular programs. Transcription factors such as Math1 and NeuroD are necessary for the production and/or survival of granule cells, with little or no effects on PCs and interneurons (1, 4). In agreement with a previous study (7), we show that *Ptf1a* null mutants exhibit cerebellar hypoplasia and a virtual loss of interneurons (labeled by Pax-2 and *GAD65/67*) and PCs. At E14, *Ptf1a* null mutants already exhibit a dramatic decrease in these neurons, indicating that the *Ptf1a* mutation led to a marked deficit in the generation of PCs and interneurons (Fig. 6). The lack of PCs and interneurons was more dramatic at E16–E18, which, together with the increase in cell death in *Ptf1a* null mutants, suggests that the few neurons that are generated die shortly after becoming postmitotic. This observation supports an additional role for Ptf1a in cell survival, which may be similar to what has been proposed for the transcription factor NeuroD in granule cells (34). We conclude that Ptf1a plays a pivotal role in cerebellar development by controlling both the generation and specification of PCs and interneurons and their subsequent survival.

***Ptf1a* and the Control of Granule Cell Fate.** Our expression and lineage analyses show that, in wild-type mice, *Ptf1a* is not expressed in granule cell progenitors in the RL or EGL or by postmitotic granule cells. Consistent with this notion, *Ptf1a* inactivation apparently does not affect the RL or EGL until at least E18, except for minor changes in cell distribution, which may be secondary to the lack of PCs (7). Using $Ptf1a^{Cre/Cre}$;*R26R* embryos, we have addressed the fate of cells that activate *Ptf1a* in *Ptf1a* null mice. Unlike in wild-type cerebella, in which β-Gal labels PCs but not the EGL, E12-born *Ptf1a*-derived cells in the mutant cerebellum populate the EGL and exhibit the cell size, shape, and fine structure typical of EGL cells. During normal development, EGL precursors originate in the RL and tangentially migrate rostrally to form the EGL (2–5). Analyses of β-Gal expression in *Ptf1a*-deficient brains showed that the rostral EGL is heavily populated with β-Gal-labeled cells and that the caudal EGL and RL are devoid of β-Gal staining, indicating that the mutant EGL is actually a mosaic of RL and VZ-derived precursors (Fig. 6). Moreover, cells derived from *Ptf1a*-active precursors in $Ptf1a^{Cre/Cre}$;*R26R* null embryos express transcription factors and differentiation markers characteristic of EGL cell precursors, such as Math-1, Zic1/2, and Reelin (26, 27, 35). Given that these genes are not expressed by PCs or interneurons at any point in their lifetime, our data indicate that neurons originating in the cerebellar VZ at E12 that would normally express *Ptf1a* and generate PCs and interneurons instead adopt a phenotype characteristic of EGL cell precursors in *Ptf1a* null mice (Fig. 6). Because the generation of EGL precursors is not a default process, but rather is regulated by dorsally expressed extracellular morphogens such as BMPs (36–38), our data would suggest that lack of Ptf1a enables VZ-derived cells to respond to such inductive signals.

Thus, in addition to its role in PC and interneuron specification and survival, the present data suggest that Ptf1a suppresses the granule cell phenotype fate of VZ progenitors, thus acting as a molecular switch that determines the fate of VZ precursors. This raises the possibility that Ptf1a may negatively regulate genes coding for transcription factors required for granule cell specification. A recent study in the spinal cord has shown that in *Ptf1a* null mutants, *Ptf1a*-derived cells, normally fated to dorsal

horn (dI4 and dIL^{A}) GABAergic interneurons, adopt a glutamatergic fate (14). A similar finding has been reported in the retina (15). Altogether, it is likely that the *Ptf1a* gene may control the expression of key regulatory genes in certain GABAergic phenotypes (such as PCs and interneurons in the cerebellum and spinal cord), although we can only speculate as to which genes are targeted by Ptf1a. For instance, Pdx1, a transcription factor that works in tandem with Ptf1a for pancreas formation, activates the *GAD67* promoter in rat islet cells (39). In conclusion, our data indicate that Ptf1a is positioned to regulate the generation of cerebellar neuronal types (i.e., GABAergic vs. glutamatergic) as well as the correct lamination pattern and neuronal numbers of the cerebellum, all of which are essential for correct cerebellar organization and function.

Materials and Methods

Ptf1a$^{Cre/+}$ mice were crossed with Gt(ROSA)26Sor^{Tm1Sor} (R26R), and double-transgenic progeny were collected at E12–P20 (1, 2). *Ptf1a* null mice (homozygous *Ptf1a*$^{Cre/Cre}$) were obtained by breeding heterozygous *Ptf1a*$^{Cre/+}$ mice. Linage Fracing of *Ptf1a*-deficient cells was performed in *Ptf1a* null embryos in a heterozygous *R26R* background. Wild-type and *Ptf1a* null mice were processed by ISH immunohistochemistry, X-Gal staining, and electronic microscopy. Cerebella were also dissected, and RNA isolation was performed. Expression analyses were performed by using the one-step RT-PCR set (Qiagen, Valencia, CA) (see SI Table 1). E12 and E18-pregnant dams were i.p. injected with BrdU, and embryos were analyzed at E18 (see *SI Methods*).

We thank R. J. MacDonald and J. E. Johnson for having shared unpublished information and for other valuable contributions; A. Skoudy, S. Maqueda, and L. Bardia for technical support; the investigators mentioned in the text for having shared reagents; and the personnel of the animal facilities at Institut Municipal d'Investigació Mèdica/Universitat Pompeu Fabra for continued support. This study was supported by Spanish Ministry of Education and Science Grants SAF2004-01137 (to F.X.R.), BFU2006-13651 (to J.A.D.R.), and SAF2005-0171 (to E.S.); National Institutes of Health Grant DK042502 (to C.V.E.W.); and grants from Marató de TV-3, Instituto de Salud Carlos III (ISCIII) (to F.X.R.), CIBERNED CB06/05/0098, and La Caixa Foundation (to E.S.). I.A. has a fellowship from the Basque government.

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